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Chronic inhalation oncogenicity study of isoprene in B6C3F$_1$ mice

Michael E. Placke[*a], Larry Griffis[b], Michael Bird[c], James Bus[d],
Ronald L. Persing[a], L. Anthony Cox, Jr.[e]

[a]Battelle Columbus Division, 505 King Avenue, Columbus, OH 43201-2693, USA
[b]Chevron Research and Technology Co., Richmond, CA, USA
[c]Exxon Chemical Co., E. Millstone, NJ, USA
[d]Dow Chemical Co., Midland, MI, USA
[e]Cox and Associates, Denver, CO, USA

Abstract

The oncogenic potential of isoprene as affected by concentration, length of daily exposure, and weeks of exposure over the life-span of the animal, as independent variables, was evaluated. Ten groups were exposed for 8 h/day, 5 days/week as follows (ppm-weeks): 0–80, 10–80, 70–40, 70–80, 140–40, 280–20, 280–80, 700–80, 2200–40, 2200–80. Two groups were exposed for 4 h/day: 2200–20, 2200–80. Groups were held until 96 or 105 weeks on study. The concentration × time (duration of exposure) values provided a series of theoretically equivalent exposure hazards. There was an exposure-related increased incidence of liver, lung, Harderian gland and forestomach tumors, and hemangiosarcomas and histiocytic sarcomas. The LOEL appeared to be 70 ppm. These results are similar to the profile of tumors seen in 1,3-butadiene (BD)-exposed mice without the early onset of T-cell lymphoma as seen with BD. Isoprene appears to be about one order of magnitude less potent than BD in mice. Statistical analyses indicated that the product of isoprene concentration, and length/duration of exposure was not a sufficient basis for predicting tumor risk at any site. Extrapolation of tumor probability between the high and low doses based on cumulative exposure was not appropriate and could not be justified by statistical models. A threshold effect level and strong nonlinearities with respect to concentration appeared to exist for tumor development in this study.

Keywords: Isoprene; Oncogenicity; Mice; Inhalation

1. Introduction

Isoprene monomer (1-methyl-1,3-butadiene) is a colorless, volatile, flammable liquid with a boiling point of 34.1°C and a vapor pressure of 493 mm at 20°C. It is industrially derived from petroleum cracking and is used in the production of butyl rubber and as a copolymer in numerous applications. Isoprene is the major endogenous hydrocarbon exhaled by humans at rates up to 250 µg/h in nonsmokers and somewhat higher rates in smokers as isoprene is present in tobacco smoke (Conkle, 1979). Isoprene is emitted to the atmosphere by many species of plants and is the

*Corresponding author. Tel: +614 424 4898.

base unit of the terpenes existing in natural rubber and in fruits such as the tomato (Bond, 1991).

Isoprene is metabolized by liver microsomal P-450-dependent mono-oxygenases resulting in the formation of two monoepoxides (Gervasi and Longo, 1990). This metabolic pathway has been confirmed with liver microsomes from rat, mouse, rabbit, and hamster (Longo et al., 1985) and the V_{max} value for metabolism to the mono-epoxide is approximately 7 times greater in mice compared to rats. The monoepoxides can be further oxidized to the diepoxide by rat and mouse liver microsomes. In vivo, it appears that substantial metabolism may occur in the lung (Dahl et al., 1987) and rats metabolize a greater fraction of inhaled isoprene than do mice (Bond et al., 1991). Formation of the diepoxide is probably crucial to isoprenes mutagenic/genotoxic activity (Peter, 1987).

Isoprene was not mutagenic in five *Salmonella* strains in the presence or absence of metabolic activation (DeMeester, 1981; Mortelmans, 1986). Both monoepoxide metabolites of isoprene were negative in *Salmonella* (TA98 and 100, without S-9) whereas the monoepoxide metabolite of 1,3-butadiene (BD) was positive in TA100. Diepoxide metabolites of both isoprene and BD were positive in both strains (Gervasi, 1985). Mice exposed to isoprene vapor at concentrations of 440 to 7000 ppm, 6 h/day for 12 days showed no evidence of increased chromosomal aberrations.

However, a significant increase in sister chromatid exchanges and of micronucleated erythrocytes occurred at all doses (Shelby, 1990).

In an inhalation study of isoprene conducted by the NTP, mice developed exposure-related increased incidences of tumors of the liver, lung, Harderian gland, and forestomach following exposure to isoprene vapor at concentrations up to 7000 ppm for 6 months with a subsequent holding period of an additional 6 months (Melnick et al., 1994). Similarly exposed rats showed no effects.

The present chronic inhalation study in mice was undertaken to better define the shape of the dose-tumor response curve for isoprene exposure. In addition, this innovative study design explored the effect of the length of daily exposure (h/day), weeks of exposure, and cumulative (lifetime) exposure on dose-response.

2. Materials and methods

Twelve groups of 50 male $B6C3F_1$ mice were exposed to 0, 10, 70, 140, 280, 700, or 2200 ppm of isoprene vapor for 4 or 8 h/day, 5 days/week for 20, 40, or 80 weeks followed by a holding period, leading to a total planned study length of 104 weeks. Female mice (50/group) were exposed to 0, 10, and 70 ppm of isoprene, 8 h/day for 80 weeks and also held for observation through Week 104.

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Test article concentration (ppm)	Experimental design group number	No. of animals	Daily exposure (h)	Number of weeks exposed
0	1A	50M/50F	8	80
10	2A	50M/50F	8	80
70	3A	50M	8	40
70	4A	50M/50F	8	80
140	5A	50M	8	40
280	6A	50M	8	20
280	7A	50M	8	80
700	8B	50M	8	80
2200	9B	50M	4	20
2200	10B	50M	4	80
2200	11B	50M	8	40
2200	12B	50M	8	80

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Selected groups of mice were removed after 20 or 40 weeks and held in the exposure room in a separate holding chamber provided with filtered room air for the duration of the 80 week exposure period. After 80 weeks all surviving animals were transferred to a holding room through Study Week 104 and then necropsied beginning in Study Week 105. Groups 7 through 12 were necropsied early (Study Week 96) due to high incidences of mortality.

Parameters used to assess toxicity during the in-life portion of the study included clinical observations and body weight (weekly for 13 weeks and then monthly), hematology evaluations (at necropsy) and micronucleus evaluations (10 mice/group at 40 and 80 weeks). Postmortem studies included macroscopic organ examinations and complete histopathology evaluations of tissues from all mice.

The test article used for this study was isoprene (CAS no. 78-79-5) supplied by the Goodyear Tire & Rubber Company (Beaumont, Texas). Controls were exposed to filtered, conditioned room air. The purity of each cylinder of isoprene was determined prior to and again after its use by GC analysis from a sample collected directly from each cylinder. All cylinders were at least 99.0% pure and the test material contained less than one percent limonene (a degradation product). Tert-butyl catechol (TBC) was added by the supplier as a stabilizer at a target concentration of 50 ppm. TBC in each cylinder was measured and did not exceed 100 ppm for the initial cylinder analyses, with a measured range of between 4.1–95.3 ppm.

2.1. Test atmosphere generation system

Test atmospheres of isoprene were generated from the liquid as an aerosol by ultrasonic nebulization followed by evaporation in a stainless steel aging plenum prior to delivery to the exposure chambers (Hazelton H1000 or H2000, 1000 or 2000 l in volume). Filtered compressed air was supplied to the ultrasonic spray nozzle. Isoprene was fed to the spray nozzle from the storage cylinder which was pressurized with N_2 at 5 psig. Room air was drawn into the aging plenum through charcoal and high efficiency particulate air (HEPA) filters and carried isoprene vapor to the exposure chambers through a common distribution manifold with subsequent dilution at each chamber to the target concentrations. The starting concentration (2200 ppm) was drawn from the manifold through thin-plate orifice flowmeters, diluted if necessary with filtered air, and delivered to each exposure chamber. Chamber environment was maintained at $22 \pm 2°C$ and at 40 to 70% relative humidity. Chamber flow was a nominal 15 air changes per h. Chamber pressure and flowrate were monitored continuously during exposures.

2.2. Test atmosphere analysis

A Miran-980 infrared spectrometer (IR) (Foxboro Company, Watertown, MA) was used to measure isoprene concentrations in the chambers and exposure room. Prior to the study, the IR was calibrated and then recalibrated every 3 to 4 months. An hourly sampling sequence was used to minimize the step change in concentration among chambers. Some chamber samples were also analyzed with a gas chromatograph (Hewlett Packard HP 5790 system with a Flame Ionization Detector), primarily to determine the concentrations of limonene (isoprene dimer).

Uniformity within the exposure chambers was verified by measuring the isoprene concentration at the front, middle, and back of each chamber level. Buildup and decay of isoprene in the chambers were rapid with T_{90} achieved in 4 to 10 min. Using an aerodynamic particle size (APS 338; TSI, Inc., St. Paul, MN), no residual aerosol was detected during vapor generation.

2.3. Experimental animals

Mice were obtained from Charles River's Portage Laboratory and were 4 to 6 weeks of age at receipt and no older than 10 weeks at the start of the study. The mice were quarantined and acclimated for 25 days pre-study. Prior to the study, sera were collected from at least 5 mice/sex and were found free of antibody titers to common murine pathogens.

Animals were individually housed in stainless steel wire-mesh inhalation cages that fit into the exposure chambers. Each cage unit was switched weekly within the chamber between the two positions used. A 12-h light/dark cycle was maintained. Mice were fed ad libitum Certified Purina Rodent Chow in pellet form during the non-exposure periods.

2.4. Hematology evaluation

Blood samples were collected using EDTA-containing tubes from the retro-orbital sinus from each animal reaching scheduled necropsy. The following parameters were measured: red blood cell count, hematocrit, hemoglobin, white blood cell count, and white blood cell differential, and morphology evaluation.

2.5. Micronucleus evaluation

Evaluations of peripheral blood were performed on 10 animals per exposure group using samples obtained the day after the last exposure for those groups ending exposure after 40 and 80 weeks. Ten controls were also sampled when an exposed group was. A slightly modified method (micronucleated polychromatophilic or orthochromophilic erythrocytes were counted) as described by Miller (1973) was used. A total of 2000 erythrocytes from each animal were evaluated. Results were analyzed using one way analysis of variance or a nonparametric test (Kruskal-Wallis and Dunn's summed rank).

2.6. Necropsy procedures

A complete gross necropsy was performed on all animals that died during the study or at scheduled necropsy. Animals were anesthetized using pentobarbital and exsanguinated. Tissues from all major organs were removed and preserved in 10% neutral buffered formalin, except for the eyes and testes which were fixed in Bouin's solution. Histopathologic evaluations were conducted on all tissues from all animals, including early death or moribund-sacrificed animals. Tis-

sues were embedded in paraffin, sectioned at approximately 5 μm, stained with hematoxylin and eosin and the slides evaluated by light microscopy.

2.7. Statistics

Statistical evaluations of body weight, organ weight, and hematology data were performed using analysis of variance (ANOVA) followed by Duncan's new multiple range test ($P \leqslant 0.05$). Incidences of specific tumor types were analyzed using Fisher's exact test which was applied repeatedly to each combination of exposure group and tumor type.

Additional statistical analyses were conducted using a variety of traditional parametric and nonparametric methods and artificial intelligence approaches (Cox, 1989; Biggs et al., 1991). These methods and results are the subject of a companion manuscript within these proceedings.

3. Results

3.1. Exposure system results

The grand means (mean of all samples collected) of the isoprene concentrations during the 80 week exposure were 10.4, 70.1, 141, 280, 700, 2173 (4 h), and 2203 (8 h) ppm. All values were within 4% of target concentrations. The grand mean values for chamber temperature ranged from 22.1 to 22.7°C and the grand mean values for relative humidity ranged from 52.3 to 55.0%.

3.2. Survival

There was an isoprene concentration-related effect on survival. At approximately Week 80, animals exposed to higher concentrations (280 to 2200 ppm, Groups 7 through 12) had lower survival rates (< 50%) than the controls or animals exposed to less than 280 ppm for 80 weeks. Groups 7 through 12 were necropsied in Week 96 to ensure an adequate number of animals with non-autolyzed tissues for histopathology.

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3.3. Clinical observations

An exposure-related increase in the incidence of swelling of the abdomen was observed as early as Day 56 in the 2200 ppm group, but no similar changes were noted in the lower exposure groups until Day 303. The frequency of abdominal swelling correlated directly with the incidence of liver tumors. Swelling of the eye was first observed on Day 534 and the incidence increased with time and concentration with the highest incidence occurring in the 2200 ppm groups. This clinical change was correlated with the incidence of Harderian gland tumors.

No other clinical changes (other than apparent tumors and associated deterioration of health) were considered to be directly related to isoprene exposure.

3.4. Body weight values

All groups gained weight throughout the first year of the study and then weights plateaued until necropsy. There appeared to be no significant effect on group mean body weight or body weight gain. Sporadic values were statistically different from control but these changes were transient and not dose-related.

3.5. Hematology results

No significant effects were observed on any of the hematological parameters.

3.6. Micronucleus results

After 40 weeks of exposure, animals in Groups 1 (control), 3 (70 ppm), and 5 (140 ppm) had similar group mean values (Fig. 1). Group 11 (2200 ppm) animals had micronucleus counts that were significantly increased (147%) compared to controls (Fig. 1).

After 80 weeks of exposure, animals in Groups 1 (control), 2 (10 ppm), and 4 (70 ppm) had similar values. All mice exposed to higher concentrations of isoprene ($\geqslant 280$ ppm) had significantly ($P \leqslant 0.05$) greater micronuclei values compared to controls. Animals in Group 7 (280

ppm), Group 8 (700 ppm), Group 10 (2200 ppm/4 h), and Group 12 (2200 ppm) were 92, 133, 167, and 125% higher, respectively, than the controls.

3.7. Necropsy results

Gross lesions considered to be directly or indirectly related to isoprene exposure were observed in the eyes, forestomach, Harderian gland, liver, lung, spleen, mesenteric lymph node, and testis of male and, in general, correlated with the histopathologic findings. Eyes were opaque and often protruded due to enlarged Harderian glands. The forestomach, when affected, had small nodules on the mucosal surface and two isoprene-exposed mice had forestomach masses. The incidences of liver nodules and masses were increased in higher isoprene exposure-level concentration groups compared to controls, as were the incidences of lung nodules and masses. The number of enlarged spleens were slightly increased at higher isoprene exposure-levels, as was the incidence of enlarged mesenteric lymph nodes. The incidence of small testes was increased at exposures above 5600 ppm-weeks (concentration times exposure-weeks). Absolute testis, testis-to-body weight ratio and testis-to-brain weight ratio values were significantly less (20 to 30%) in Groups 7, 8, 10, and 12 compared to controls.

* Statistically different from control ($p \leq 0.05$)
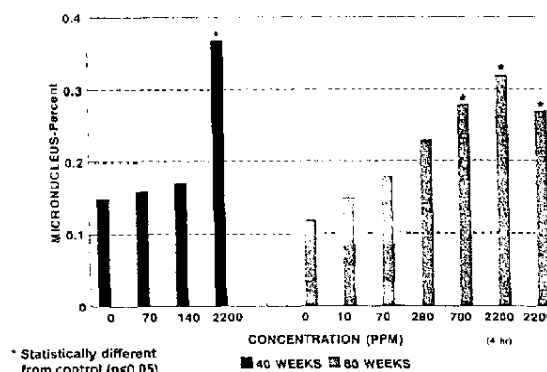■ 40 WEEKS ▨ 80 WEEKS

Fig. 1. Micronucleus results.

No increase in probable tumors was discernible grossly in isoprene-exposed females. Heart weight was significantly less in 10 ppm females but not at 70 ppm. Mean ovary weight was less, although not significantly, in 10 and 70 ppm females compared to controls. The lower gonadal weights may have been exposure-related.

3.8. Histopathology results

3.8.1. Neoplastic changes

Several types of neoplasms occurred only, or in greater frequency, in mice exposed to isoprene compared to controls.

3.8.1.1. Male mice. Isoprene exposure caused an increase in histiocytic sarcomas and in neoplasms of the liver, lung, Harderian gland, and forestomach of males (Table 1). The incidence of hepatocellular adenomas and carcinomas were increased (significantly at exposure concentrations $\geqslant 140$ ppm), as were hemangiosarcomas

and histiocytic sarcomas of the liver. Metastases of hepatocellular carcinomas to the lung were also more prominent in animals receiving higher exposures. Some of these primary and metastatic liver tumors appeared to be more anaplastic and aggressive in their growth as compared to the spontaneous liver tumors in controls.

Primary alveolar/bronchiolar adenomas and carcinomas were significantly increased in incidence in Groups 8, 11, and 12. Several lung carcinomas in exposed mice were locally invasive to the mediastinal and thoracic area, and in five cases metastasized to the liver. The lung was also the metastatic site for Harderian gland carcinomas, and two metastatic squamous cell carcinomas from the forestomach. Histiocytic sarcoma of the lung was also slightly more prevalent in isoprene-exposed mice than in controls.

The incidence of Harderian gland adenomas was significantly increased as the isoprene exposure concentration increased. Harderian gland carcinomas were not as numerous as adenomas but were present in the higher concentration

Table 1
Summary of isoprene exposure-related neoplasms in male mice

Group	1	2	3	4	5	6	7	8	9[a]	10[a]	11	12
(ppm/weeks)	0/80	10/80	70/40	70/80	140/40	280/20	280/80	700/80	2200/20	2200/80	2200/40	2200/80
(ppm × weeks)[a]	0	800	2800	5600	5600	5600	22 400	56 000	22 000	88 000	88 000	176 000
Alveolar/bronchiolar												
Adenoma	11/50	16/50	8/50	4/50	10/50	16/50	13/50	23/50	14/50	15/50	29/49	30/50
Carcinoma	0	1	0	2	1	3	1	7	2	3	3	7
Hepatocellular					*	*	*	*	*	*	*	*
Adenoma	11/50	12/50	14/49	15/50	22/50	18/49	24/50	27/48	22/50	21/50	28/47	30/50
Carcinoma	9	6	11	9	10	12	16	17	12	15	18	16
Harderian gland					*	*	*	*	*	*	*	*
Adenoma	4/47	4/49	13/48	9/50	12/50	16/49	17/50	26/49	19/49	28/50	31/49	35/50
Carcinoma	0	0	0	0	2	3	1	3	1	2	0	2
Hemangiosarcoma												
Heart	0/49	0/50	0/49	0/50	0/50	0/50	2/50	1/50	4/50	1/50	1/49	1/50
Spleen	1/49	3/48	1/47	2/50	3/50	2/47	1/50	2/48	2/48	2/50	0/47	1/49
Forestomach												
Squamous papilloma	0/50	0/48	0/47	0/50	0/49	0/46	0/50	1/47	0/48	1/50	2/47	1/50
Squamous carcinoma	0	0	0	0	0	0	1	0	1	1	0	3
Histiocytic sarcoma	0/50	2/50	2/50	2/50	1/50	8/50*	4/50	2/50	5/50	7/50*	7/50*	2/50
Any lymphoma	2/50	1/50	2/50	4/50	1/50	7/50	5/50	4/50	4/50	4/50	5/50	6/50

[a]Groups 9 and 10 were exposed for 4 h/day instead of 8 h/day.
*Incidence is significantly greater than the control group ($P < 0.05$).

Table 2
Summary of isoprene exposure-related neoplasms in female mice

Group	1	2	4
(ppm/weeks)	0/80	10/80	70/80
(ppm x weeks)	0	800	5600
Alveolar/bronchiolar			
Adenoma	5/50	6/50	5/50
Carcinoma	0	0	0
Hepatocellular			
Adenoma	4/50	6/50	3/50
Carcinoma	2	4	2
Harderian gland			
Adenoma	2/49	3/49	8/49*
Carcinoma	0	0	0
Hemangiosarcoma			
Heart	0/50	0/50	0/50
Spleen	1/50	1/49	4/50
Forestomach			
Squamous papilloma	0/50	0/49	0/50
Squamous carcinoma	1	0	0
Pituitary adenoma	1/49	6/46	9/49*
Histiocytic sarcoma	4/50	5/50	6/50
Any lymphoma	9/50	10/50	12/50

*Incidence is significantly greater than the control group (P < 0.05).

groups. These carcinomas were diagnosed generally by the presence of foci or nodules of Harderian gland cells in the lung parenchyma or by evidence of Harderian cells in veins or lymph vessels leaving the tumor.

Squamous cell carcinomas of the stomach were present in six mice exposed to over 5600 ppm-weeks total exposure. Most were highly invasive locally and two metastasized to the lung. Squamous papillomas of the forestomach were found in the four highest exposure groups (8, 10, 11 and 12) of male mice.

Exposed mice also had a slightly increased incidence of hemangiosarcomas in the spleen and heart compared to controls. Cardiac hemangiosarcomas are rare in B6C3F1 mice. A large compendium of historical tumor incidences lists no cardiac hemangiosarcomas among 658 control B6C3F1 mice on recent 2-year inhalation studies.

3.8.1.2. Female mice. Female mice were exposed to lower concentrations of isoprene than males. Neoplastic lesions that may have been exposure-related were hemangiosarcomas in the spleen, Harderian gland adenomas, and adenomas of the pituitary gland (pars distalis) (Table 2). Since the number of actual neoplasms in these organs was small in the female mice, historical incidences were considered (NIEHS, Research Triangle Park, NC). In 654 control mice from various inhalation studies, the number of splenic hemangiomas and/or hemangiosarcomas was four (0.61%), suggesting that the four hemangiosarcomas in the 70 ppm females (8%) may have been related to isoprene exposure. From the same data set, the historical incidence of Harderian adenomas was 22/661 (3.33%), with a range of 0 to 16% and the historical incidence of pituitary adenomas was 127/629 (20.19%), with a range of 2 to 44%. Thus, the relationship of these latter two neoplasms to the isoprene exposure is only equivocal, considering the variability in the incidence of these lesions.

3.8.2. Non-neoplastic changes

Numerous non-neoplastic lesions were observed in both isoprene exposed and control mice, and some lesions were increased in incidence relative to controls. There was a slight increase in alveolar epithelial lining cell hyperplasia at the higher doses in male mice. Alveolar hyperplasia could be a precursor of alveolar-bronchiolar adenoma which was significantly increased in exposed males.

Focal areas of epithelial hyperplasia of the forestomach mucosa were observed more often in males exposed to higher levels of isoprene. This hyperplasia could represent an early adenomatous change in the forestomach (a "pre-neoplastic" change).

The nose/nasal cavity had a significant non-neoplastic lesion that was more prominent in the four highest doses in males and the highest dose in females. The lesion was in the dorsal meatus and consisted of focal areas of mild metaplasia of the olfactory epithelium to respiratory epithelium. The metaplastic epithelium often invaginated to form glandular patterns.

Several degenerative lesions were seen at higher incidence in exposed mice. These included chronic degeneration of the interventricular septal myocardial muscle, seminiferous cell atrophy in the testes, increased sperm granulomas in the epididymis, and chronic-active inflammation of preputial glands in higher dose males. The reasons these degenerative changes occurred at a higher incidence were unclear, but may reflect the relatively poorer condition of exposed mice due to other complicating health conditions (tumors).

Hematopoietic cell proliferation in the spleen and myeloid hyperplasia of the bone marrow was increased slightly in all isoprene-exposed mice of both sexes. This may reflect a direct effect of isoprene exposure on the erythron, or may be an indirect effect, in that increased hematopoiesis is frequently noted morphologically in stressed mice, particularly in mice with malignant neoplasms. There were no significant changes in red blood cell parameters.

3.8.3. Biostatistical analyses

In addition to standard statistical analysis, the tumor incidence data were further explored using more elaborate methods to analyze relationships between exposure parameters and particular tumor incidences. The results of these analyses are the subject of a separate manuscript (Cox et al.) published in these proceedings, but a few are summarized here.

There was no evidence of a lymphoma response analogous to the one for 1,3-butadiene in mice. The dramatic, relatively early increase in lymphocytic lymphomas at high concentrations of 1,3-butadiene (Melnick et al., 1990) has no parallel in the isoprene study, and the association between isoprene exposure and lymphomas in male mice was relatively weak.

Cumulative exposure was not an adequate predictor of tumor incidence. The same cumulative exposure appeared more or less toxic, depending on how it was administered over time. For example, Group 6 (280 ppm, 20 weeks) had higher lung tumor incidence than Group 4 (70 ppm, 80 weeks). Increasing concentration and proportionately decreasing weeks of exposure appeared to increase lung tumor incidence, rather than leaving it unchanged. The liver tumor data are consistent with this pattern; doubling exposure hours-per-day but halving total weeks of exposure appears to increase lung and liver tumor incidence, rather than leaving them unchanged.

Doubling the weeks of exposure, while leaving concentration the same, less than doubled the incidences of liver and lung tumors (Groups 3 versus 4). Even quadrupling weeks of exposure from 20 to 80 did not quadruple tumor incidences, but had only a small effect on incidence (Group 6 vs. 7 and Group 9 vs. 10). In contrast, doubling hours-per-day of exposure appeared to significantly increase tumor incidence (Groups 10 vs. 12). The ratio of lung tumor incidence to liver tumor incidence was significantly different in different dose groups, showing that the probabilities of tumors at different anatomic sites depended on the dose factors in different ways. The traditional multistage risk model, as it is usually applied, cannot easily account for these observations.

Tumors at different anatomic sites did not occur statistically independently of each other. For example, liver adenomas and lung adenomas were significantly positively associated, especially at higher concentrations. This means that, when other factors (e.g. exposure concentration and duration) were the same, then a randomly selected mouse was more likely to have one of these tumors if it also had the other.

In this study, 10 ppm was a NOEL (no observed effect level) for carcinogenic effects of isoprene among the $B6C3F_1$ mice examined. The LOEL (lowest observed effect level) for tumors in this study was between 70 ppm and 140 ppm.

4. Discussion and conclusions

Exposure to the isoprene concentrations and varied schedules used in this study did not produce any clinically important signs of general toxicity. The clinical health of the animals deteriorated in relation to tumor development (particularly malignancies). There was a positive correlation with isoprene exposure and clinically evident tumors.

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The significant outcomes in this study related to isoprene exposure (concentration and duration) were increases in selected tumor development and the associated mortality that occurred as a result. The mortality rate increased significantly after approximately 90 weeks on study in mice exposed to 280 ppm for 80 weeks and all higher exposures. Survival was near or below 50% in these groups by study week 95, at which time the surviving animals were necropsied. This high mortality was associated with a greater incidence of tumors. Survival in all other groups was generally above 60% after 2 years on study.

Exposure to isoprene in this study resulted in genotoxic effects with the mean incidence of micronuclei in peripheral blood significantly ($P \leq 0.05$) greater at 700 ppm or higher after 80 weeks. Similar effects were observed after 40 weeks, although only the 2200 ppm animals had significant elevations of micronuclei compared to the control, 70, and 140 ppm groups. The 280 and 700 ppm groups were not sampled at the 40 week point by protocol design. There was no apparent relationship to exposure duration or cumulative exposure on the incidence of micronuclei. Exposure to 1,3-BD also increases micronuclei in mice.

A partial hindlimb paralysis and spinal cord degeneration was reported in mice exposed to 70 ppm for 6 months in the NTP isoprene study (Melnick et al., 1994). In contrast to that report, there were no apparent effects on motor function and no exposure-related lesions in the spinal cord in this study after much higher cumulative isoprene exposures.

A variety of neoplasms were diagnosed in both isoprene-exposed and control mice of both sexes. However, several types of neoplasms occurred only, or in greater frequency, in mice exposed to isoprene. Isoprene exposure caused an increase in neoplasms of the lung, liver, Harderian gland, forestomach and lymphoreticular system (histiocytic sarcomas) of male mice and in the Harderian and in the pituitary gland of female mice at exposure concentrations at or above 70 ppm.

Female mice were exposed to lower concentrations of isoprene than males, the highest exposure being 70 ppm for 80 weeks. The only neoplastic lesions that may have been exposure-related in females were hemangiosarcomas in the spleen, Harderian gland adenomas, and adenomas of the pituitary gland (pars distalis).

Concentration and duration of exposure did not affect tumor risk symmetrically. Nor did length of daily exposure and duration of exposure (weeks) influence tumor risk in equivalent proportions. For example, increased isoprene concentrations of a factor of 4 (from 70 ppm to 280 ppm), with a concurrent reduction in exposure duration by an equal factor of 4 (from 80 to 20 weeks) resulted in a significant increase in lung tumors from 12% to 38% and the liver tumor incidence increased 25%, despite the equivalent cumulative exposure values. Further, exposure to a common concentration of 2200 ppm, for 4 h/day for 80 weeks compared to 8 h/day for 40 weeks resulted in significantly different tumor incidences. The lung tumor rate nearly doubled in the 8 h/day group, but this group had a lower incidence of histiocytic sarcoma.

The results from this study, including the target organs and tumor types, were similar to that seen in mice after exposure to 1,3-butadiene. However, butadiene appeared to be more potent by about one order of magnitude with a low observed effect level (LOEL) of 6 ppm, while isoprene had an apparent LOEL of 70 ppm in this study. Since only the diepoxide of isoprene is genotoxic, the results of this isoprene bioassay contribute to evidence suggesting that the similar effects of 1,3-butadiene are likely due to the bifunctional diepoxide metabolites.

There appeared to be a well delineated threshold for oncogenic effects following isoprene exposure in mice, which varied slightly by organ and tumor types. For male mice, the LOEL appeared to be 700 ppm for lung tumor and hemangiosarcoma, 280 ppm for malignant forestomach tumors and histiocytic sarcomas, 140 ppm for liver tumors, and 70 ppm for Harderian gland tumors. For female mice the LOEL appeared to be 70 ppm for total non-liver, non-lung adenomas and possibly for hemangiosarcomas.

These results indicate that concentration, length of daily exposure, and weeks of exposure

did not affect tumor incidence equivalently and total cumulative exposure was not sufficient for predicting oncogenic risk from isoprene exposure in mice. For exposures of at least 20 weeks' duration, isoprene concentration was more important in affecting the tumor response than was weeks of exposure. An important question raised by these results is what is the minimum duration of exposure, less than 20 weeks, which would cause an observable oncogenic response in mice from isoprene? This same question is also relevant to 1,3-butadiene in mice. It has been reported that a one-day exposure to butadiene at concentrations of 1000, 5000, and 10000 ppm did not increase the tumor incidence in exposed mice (Bucher et al., 1994).

Isoprene, unlike 1,3-butadiene, is a known endogenous metabolite in animals. The isoprene concentrations in this study were substantially higher than endogeneously-generated alveolar air concentrations which are estimated to be about 0.2 ppm (Peter et al., 1990). A practical threshold should exist for carcinogenic risk from isoprene, and this should be incorporated into the assessment of risk from external exposures to isoprene.

The results of this study may have important implications for both the theoretical framework and the practice of quantitative cancer risk assessments. They also suggest that experiments may be designed to uncover aspects of cancer dose-response relations that may not always be as clearly seen in the traditional 2-year bioassay designs.

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